Archives of the Balkan Medical Union

vol. 52, no. 3, pp. 291-297
September 2017

ORIGINAL PAPER

RECREATIONAL ANGLER EXPOSURE TO DOMOIC ACID VIA CONSUMPTION OF CONTAMINATED SHELLFISH FROM THE BLACK SEA, BULGARIA: A PRELIMINARY STUDY

Zlatina V. Peteva[1], Stanislava Georgieva[1], Mona Stancheva[1], Lubomir Makedonski[1]

[1] Department of Chemistry, Medical University „Prof. Dr. P. Stoyanov"- Varna, Bulgaria

Abstract

Introduction. Domoic acid (DA) is a neurotoxin that causes amnesic shellfish poisoning with gastrointestinal symptoms (vomiting, diarrhoea or abdominal cramps) and/or neurological symptoms (confusion, loss of memory, or other serious signs such as seizure or coma). Shellfish are recognized vectors of DA to humans. However, the exposure of anglers in Bulgaria through consumption of DA-contaminated shellfish is unknown.
The aim of this study was to measure DA in shellfish species *Mytilus galloprovincialis* targeted by anglers in North Black Sea, Bulgaria and to assess anglers exposure regarding their shellfish consumption patterns.
Materials and methods. The research is conducted in the period September 2016 – May 2017. DA was confirmed via a method for the simultaneous identification and quantification by liquid chromatography–tandem mass spectrometry (LC–MS/MS). An intercepted survey, a type of face-to-face interview, was conducted to determine whether recreational anglers were at risk of exposure to DA toxins as a result of their consumption of harvested shellfish.
Results. Mean DA concentration is 0,707 μg DA/g hepatopancreas which is much less than the current EU limit of 20 mg DA/kg shellfish meat. Estimated

Résumé

Exposition des pecheurs amateurs à l'acide domoique par la consommation de crustacés contaminés de la Mer Noire, Bulgarie: étude préliminaire

Introduction. L'acide domoïque (DA) est une neurotoxine qui provoque une intoxication aux crustacés amnésiques avec des symptômes gastro-intestinaux (vomissements, diarrhée ou crampes abdominales) et / ou des symptômes neurologiques (confusion, perte de mémoire ou autres signes graves comme la crise ou le coma). Les crustacés sont des vecteurs reconnus de DA chez les humains. Cependant, l'exposition des pêcheurs à DA par la consommation de crustacés contaminés dans la mer de la Bulgarie est inconnue.
L'objectif de cette étude était de mesurer DA dans les espèces des coquillages *Mytilus galloprovincialis* ciblées par les pêcheurs du nord de la mer Noire, en Bulgarie et d'évaluer l'exposition des pêcheurs à la ligne concernant leurs habitudes de consommation de crustacés.
Matériaux et méthodes. La recherche s'est déroulée au cours de la période septembre 2016 – mai 2017. DA a été confirmé par une méthode pour l'identification et la quantification simultanées par chromatographie liquide-spectrométrie de masse en tandem (LC-MS / MS). Un sondage d'interception, un type d'entretien

Corresponding author: Zlatina Veselinova Peteva
Marin Drinov 55, 9002 Varna, Bulgaria
e-mail: zlatina_peteva@mail.bg

acute dietary exposure of 1.417 µg DA/ kg bw does not exceed the acute reference dose of 30 µg DA/ kg bw
Conclusion. The present study showed that anglers cannot be exposed to symptomatic DA doses through consumption of their catch.

Key words: domoic acid, shellfish consumption, recreational anglers, exposure assessment.

Abbreviations
DA – domoic acid
ASP – amnesic shellfish poisoning
EU – European Union
LC- Liquid chromatograph
MS- mass spectrometry
bw – bodyweight
AE- acute exposure
ArfD – acute reference dose
hp – hepatopancreas

face-à-face, a été mené pour déterminer si les amateurs de la pêche risquaient d›être exposés à des toxines DA en raison de leur consommation de crustacés récoltés.
Résultats. La concentration moyenne de DA est de 0,707 µg de DA / g d'hépatopancréas, ce qui est bien inférieur à la limite actuelle de l'UE de 20 mg de DA / kg de crustacés. L'exposition alimentaire aiguë estimée est de 1.417 µg DA / kg pc et ne dépasse pas la dose de référence aiguë de 30 µg DA / kg pc.
Conclusion. La présente étude a montré que les pêcheurs ne peuvent pas être exposés à des doses de DA symptomatiques par la consommation de leurs prises.

Mots-clés: acide domoïque, consommation de crustacés, amateurs de pêche, évaluation de l'exposition.

Introduction

Domoic acid (DA) is a naturally produced algal toxin that is responsible for a human illness known as amnesic shellfish poisoning (ASP). This neurotoxic illness was first identified in 1987, when more than 143 people became ill and 4 died after consuming DA-contaminated mussels harvested from cultivation beds on the eastern coast of Prince Edward Island, Canada[1,2,3].

Clinical signs of ASP in humans consist of gastrointestinal distress, confusion, disorientation, seizures, permanent short-term memory loss, and in the most severe cases death[3].

DA was reported in Bulgaria in 2011. Bivalve mollusks from mussel farms were analyzed. In some samples, domoic acid was in the range of 0.02 – 0.55 mg/kg shellfish meat, which is not harmful to consumers, because these quantities are significantly lower than the safety limit[4]. No ASP cases have been reported in Bulgaria.

DA is produced by diatoms *Pseudo-nitzschia*, mostly[5], and by marine red algae of the genus Chondria[6], and has been reported to accumulate in a wide variety of seafood, including mussels. DA is heat stable and cooking does not destroy the toxin. However, normal home cooking processes, such as boiling and steaming, could reduce the amount of DA in shellfish meat, due to partial leaching of the toxin into the cooking fluids[6] .

While the evaluation of the DA concentration is performed in many countries on a regular basis, the exposure assessment of consumers to DA, which relies both on reported occurrences and representative consumption data, is scarce. The limited consumption data pose a considerable breach in evaluating the exposure risk of toxins present in shellfish products. Enforcement in the European Union (EU) of the regulatory limit for DA of 20 µg/kg[7] implies that samples with DA levels below this value would be released on the market and get into the food chain[7,8]. Consumption of these products may not result in an acute intoxication with DA, but it is not yet clear what would be the effect of their long-term consumption.

According to Bulgarian Executive Agency for Fisheries and Aquaculture[9], three are the main sources from which households could source fish and other aquatic organisms, as follows: member/ members of households engaged in fishing; from shops and markets; received as a gift. Data show that households mainly source fish and other aquatic organisms from stores and markets – 86.2%. However, products from both manufacturers (mussel farms) and catches come under the trade.

In 2014, the mussel catch has a significant increase, as it was 51.5% more than in 2013 and reached 16.23 tones. A recent research showed that highest Black Sea mussel catch in Bulgaria is in the marine areas near Galata – 28 % of the total catch. In the area near Balchik, the catch is 12% of the total catch[10].

The aim of this study was to measure DA in shellfish species *Mytilus galloprovincialis* targeted by anglers in North Black Sea, Bulgaria, and to assess anglers exposure regarding their shellfish consumption patterns.

We focused the present study on the North Black Sea coast of Bulgaria, because it supports a sizeable recreational fishery and is easily accessed by anglers. Additionally, it is a site where mussels are harvested and toxic blooms of *Pseudo-nitzschia* might be frequent.

Materials and methods

Angler survey

We conducted an intercepted survey of anglers to determine whether they were at risk of exposure to DA toxins as a result of their consumption of harvested mussels. The survey was modelled after the Santa Monica Bay, San Francisco Bay and Santa Cruz Wharf seafood consumption studies[11,12,13].

We analyzed the variation in mussels DA levels across beaches to determine if consumers who harvest from different beaches should be analyzed separately or together.

An intercepted survey, a type of face to- face interview, is the most effective survey method for this context because it affords more complete coverage of anglers, including those who lack a telephone or permanent address. Intercepted surveys are the only way to reliably identify and sample the population. In addition, this facilitates the collection of more accurate and reliable data, because it allows the researcher to: clarify questions and responses, make on-site observations that contribute to more accurate and consistent identification of anglers' catch[14].

The survey consisted of 3 components: (1) an initial contact with potential interviewees to determine willingness to participate; (2) determination of spatial patterns of angler use; and (3) a survey questionnaire, administered as an in-person interview. The survey aims to determine the proportion of anglers that consume their catch.

The survey was conducted by a team of two persons: one interviewer using a questionnaire and one processing and systemizing the answers. Each interview lasted 10 to 20 minutes and occurred once or twice a month in the survey period. Survey data were processed and analyzed to be quantified the anglers who reported consuming their catch, their weight and weight of portion consumed per meal.

The survey participants were given explanations regarding the purpose and the importance of the study, in which they freely agreed to join this study.

Sample collection

In parallel with the angler survey, mussels *Mytilus galloprovincialis* from the angler catch were sampled at various locations on the North Black Sea coast, Bulgaria and collected once or twice a month. The samples were kept in cooling boxes for approximately 1 to 3 hours and transported to the laboratory. In the laboratory, samples were frozen in –18°C until further analysis.

Five samples were prepared as follows: mussel meat was separated from the shell and the digestive gland was dissected. Digestive glands were afterwards homogenized.

Sample preparation, extraction and DA concentration determination

The method for extraction and determination of domoic acid was described by Krock et al[15]. 4 g digestive gland (hepatopancreas) were homogenized subsequently with 90% and 80% methanol. The extracts were further degreased with n-hexane. The extracts were filtered through a 0.45-μm pore-size PTFE syringe filter. The resulting filtrate was transferred into an LC autosampler vial for LC–MS/MS analysis.

Mass spectral experiments were performed on AB-SCIEX-4000 Q Trap, triple quadrupole mass spectrometer equipped with a TurboSpray® interface coupled to an Agilent model 1100 LC. The LC equipment included a solvent reservoir, in-line degasser (G1379A), binary pump (G1311A), refrigerated autosampler (G1329A/G1330B), and temperature-controlled column oven (G1316A).

Statistical analysis

Data obtained were analyzed by using Microsoft Office Excel 2010. The statistical analysis of the data was based on the comparison of average values by a t-test and a significance level of $p<0.05$ was used.

Results

Angler consumption patterns

A total of 16 anglers surveyed at the North Black Sea coast, Bulgaria (Table 1). They were interviewed about their body weight and age, where do they harvest, if they consume their catch and what is the size of mussel meat portion (in gram) they consume.

Table 1. Locations and amount of anglers surveyed

Location	Amount of anglers	Mean body weight, kg
Asparuhovo	2	86.3
Balchik	2	89.5
Krapets	4	92.3
Shabla	2	93.0
Galata	6	84.0

All interviewees reported the location of mussel harvesting. 100% reported consuming their catch. Mean body weight of anglers surveyed is 89.0 kg. They all are male, aged 34-58. A similar male subpopulation studied by Ferriss et al.[16] aged 41-60 and weighs 92.9 kg.

Domoic acid in mussels

LC/MS-MS analysis revealed that domoic acid was present in all mussel samples. The domoic acid concentrations found in the different samples of mussels can be found in Table 2. Mean domoic acid concentration was found 0.707 µg DA/g hp.

Table 2. Domoic acid levels

Food	Mean concentration µg DA/g hp	Range µg DA/g hp	Positive samples	Total samples
Mussels	0.707	0.493 – 0.919	5	5

Highest domoic acid concentration was found in samples from Krapets and Shabla with values resp. 0.919 and 0.908 µg DA/g hp (Figure 1).

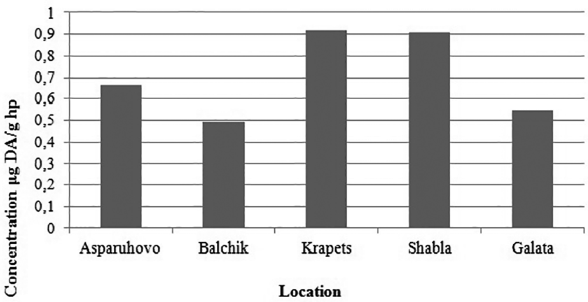

Figure 1. Concentration of domoic acid in mussel samples by location

Temporal distribution of domoic acid is presented on Figure 2. By the end of April and beginning of May, an increase in the domoic acid content is observed. Temporal and spatial concentrations of DA showed both a low correlation (resp. R^2 = 0.0215 and R^2= 0.0123). As a result, a mean temporal and location DA concentration could be used for exposure assessment.

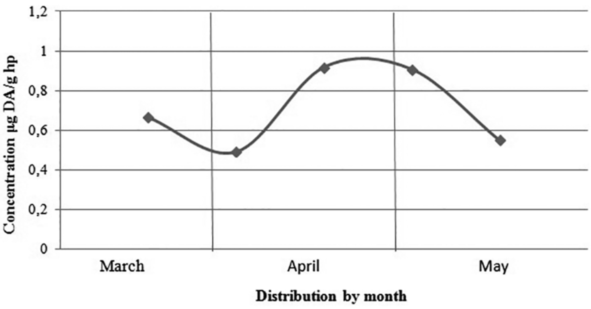

Figure 2. Temporal distribution of domoic acid

To assess the DA positive concentration (representing the concentration above the legislative norm of 20 mg/kg)[7], results should be recalculated from µg DA/g hp to mg DA/ kg shellfish meat.

Two approaches are suitable:

(1) Represent the DA hepatopancreas concentration as DA whole shellfish meat concentration. These steps are followed:

- Measure the weight of shellfish meat
- dissect the hepatopancreas and measure its weight
- analyze the DA concentration in hepatopancreas
- calculate DA concentration for the whole shellfish meat measured

Results were calculated in the range 0.0029-0.0081 mg DA/kg shellfish meat.

(2) According the Scientific Opinion of the Panel on Contaminants in the Food Chain [6] a factor of 5 was used to convert the value to whole shellfish meat. Using this method DA concentrations were calculated in the range 0.000099 – 0.000184 mg DA/ kg shellfish meat.

Results are summarized in Table 3.

Using the two approaches recalculated DA concentrations are beneath the legislative norm of 20 mg/kg. Even though in our opinion more suitable is the first approach as the digestive gland accumulates the domoic acid[17] and the determined values of concentrations are more representative.

Acute exposure to domoic acid

For the purposes of this study, acute exposure was defined as exceeding the ARfD (acute reference dose). For the estimation of daily acute exposure, mean consumption pattern calculated from the consumption database, is multiplied with mean domoic acid level from the concentration database for mussels and expressed per kg bodyweight. This probable assumption is made because a person eating shellfish will not eat the same portion size containing the same level of toxin each time. Results (Table 4) showed a mean acute exposure intake of 1.417 µg DA/kg bw which is beneath the acute reference dose of 30 µg DA/ kg bw[6]. The highest acute exposure intake of 2.442 µg DA/kg bw is in Shabla.

The weight of the lightest participant in the survey is 79 kg. The estimated DA concentration for this location is 0.908 µg DA/g hp. The calculated acute exposure is 0.843 µg DA/kg bw.

The largest portion reported in the survey comprises 300 g. This portion size is reported in three locations. The estimated acute exposures of the individuals are summarized in Table 4. All values are beneath the threshold of 30 µg DA/kg bw[6] .

Table 3. Summary of recalculated DA concentrations in accordance with the legislative unit mg DA/kg shellfish meat

Location	Approach 1 mg DA/kg shellfish	Positive concentration (above 20 mg/kg)	Approach 2 mg DA/kg shellfish meat	Positive concentration (above 20 mg/kg)
Asparuhovo	0.008140	no	0.000133	no
Balchik	0.005816	no	0.000099	no
Krapets	0.004595	no	0.000184	no
Shabla	0.005271	no	0.000182	no
Galata	0.002903	no	0.000110	no
Mean	0.005345	no	0.0001416	no

Table 4. Summary characteristics of the surveyed subpopulation and acute exposure intake values of DA

Location	mean body weight, kg	mean portion, g	acute exposure, µg DA/kg bw
Galata	86.3	166.7	1.062
Balchik	89.5	150.0	0.827
Krapets	92.3	175.0	1.743
Shabla	93.0	250.0	2.442
Asparuhovo	84.0	150.0	1.189
mean	**89.0**	**178.3**	**1.453**

Table 5. Individual acute exposures when consuming 300 g portion

Location	Acute exposure, µg DA/kg bw	ARfD, µg DA/kg bw
Galata	1.795	30
Krapets	2.842	30
Shabla	2.899	30

DA concentration expressed as µg DA/ kg shellfish meat (Approach 1 and 2) gives lower values for acute exposure intake as expressed µg DA/ g hp (Figure 3).

These results are even more encouraging, because not only the digestive gland but whole mussel meat is consumed. The low acute exposure levels on basis whole shellfish meat even more could be interpreted as an additional favorable characteristic of mussels as healthy food.

Discussion

The goal of the present study was to determine whether or not anglers were exposed to DA through

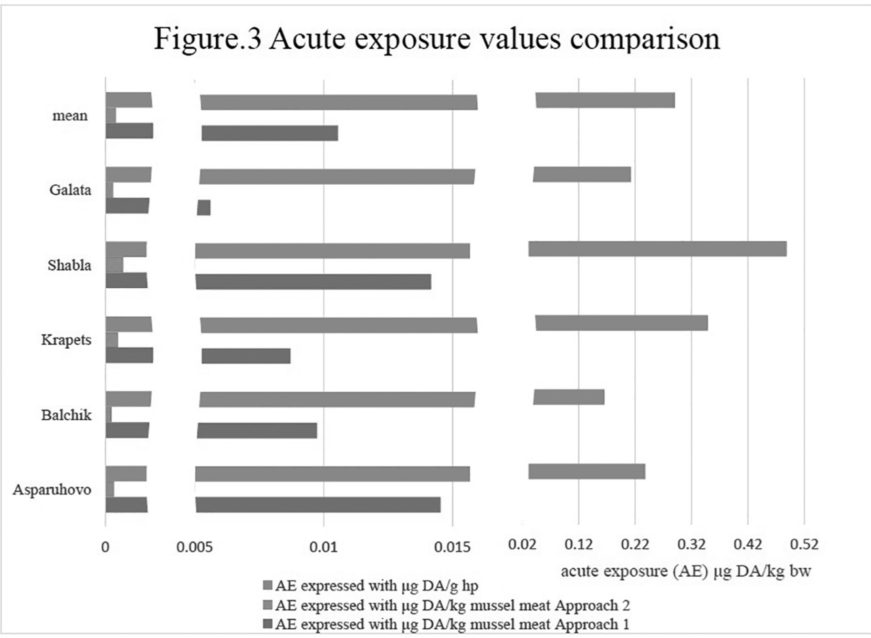

Figure 3. DA acute exposure values comparison

the consumption of their catch, since shellfish are DA vectors to their aquatic predators.

To accomplish this goal, the DA concentration in shellfish species *Mytilus galloprovincialis* targeted by anglers should be measured.

In Spring 2017, mussel samples from recreational harvesters catch were investigated for domoic acid content. All the samples were positive for DA. A similar study in Belgium in 2004–2009 showed that the percentage of the mussels positive samples was 7%[18]. A study in Croatia revealed that, of those species in the period from 2006 to 2008, domoic acid was the most prevalent marine biotoxin in the mussel *Mytilus galloprovincialis*[19].

After the first reported and severe poisoning with amnesic shellfish toxins in 1987 in Canada, there were other episodes with less severe outcomes. Reports presented levels of domoic acid in razor clams and scallops even up to 230 and 2900 ppm, respectively[20]. According to these occasional cases, EFSA stated that less than 1% of the population would be at risk for exceeding the acute reference dose. This was confirmed for Belgian population[16]. The maximum ASP levels, observed on rare occasions in Portuguese shellfish, would cause an intake of just 16 mg DA with a 100 g meal[21]. It is not surprising that ASP outbreaks are rare among the Portuguese population.

In the framework of the national monitoring program for marine biotoxins in bivalve molluscs in Greece in 2002, 83% of all samples examined contained less that 1 µg DA/g, while in 2003 this percentage was 95%. In both years, DA was only detected in springtime without any sample exceeding the regulatory level[22].

In order to protect high consumers against acute effects of marine biotoxins, the CONTAM Panel of EFSA[6] identified 400 g of shellfish meat as an appropriate estimate of a large portion size consumed in Europe to be used in the risk assessments. According to the CONTAM panel for a 60 kg adult, the chance of exceeding a dietary exposure of 1.8 mg sum DA, corresponding to the ARfD of 30 µg sum DA/kg bw, is about 1%, when consuming shellfish currently on the European market.

Non-standard pathways, leading to excessive exposure to contaminants and toxins, include high consumption rates of self-caught fish/shellfish, minorities, low-income populations, and recreational fishers[23,24,25]. Most fisheries are managed for acute (short term, high level) exposure and not chronic (long term, low level) exposure to seafood toxins, primarily due to a lack of knowledge on long-term consumption habits and associated health effects[5].

This study produced the first attempt for quantitative estimates of acute human exposure to DA from consuming mussels originated from the North Bulgarian coast catch. A subpopulation of recreational shellfish harvesters was identified to be potentially at risk to DA toxicity due to unknown exposure to DA.

In accordance with these estimated incidents of exposure not exceeding the ARfD, there have been no documented cases of amnesic shellfish poisoning reported in Bulgaria. This safety margin should be increasingly tested in the future, as toxic algal blooms are predicted to increase in frequency under future ocean warming conditions, and data on the availability of DA producing organisms in studied area.

Conclusions

Current management guidelines for human DA exposure via mussels are based on consumption rates and bodyweights that do not reflect the diverse recreational harvesting community. The inclusion of demographically and temporally specific data in DA exposure calculations resulted in the identification of recreational mussel harvester groups with estimated acute DA exposures lower than the current ARfD. No ASP cases are reported in Bulgaria, but still there is a probability of potentially unrecognized and unreported symptoms.

Calculated results for DA concentration in mussel samples from the anglers catch from North Black Sea Bulgarian coast are in the range 0.493 – 0.919 µg DA/g hp. Recalculated DA levels using two different approaches are resp. 0.005345 and 0.0001416 µg DA/kg shellfish meat and do not exceed the legislative norm.

The determined mean acute exposure intake of surveyed recreational anglers is 1.417 µg DA/kg bw, which is beneath the acute reference dose of 30 µg DA/ kg bw[6], according to the Scientific opinion of the panel on contaminants in the food chain.

The extent of this exposure to chronic low level exposure across the diversity of mussel harvesters can be determined by a more intensive, structured study that ensures adequate representation of all consumer groups. It will become increasingly important to understand and communicate the limits of environmental safety regulations, as ocean conditions change and toxic algal blooms become more frequent[26].

Acknowledgments

This study is financed by Science Fund of Medical University Varna, Project Incoming number 16012/2016.

References

1. Bates SS, Bird CJ, de Freitas ASW, et al. Pennate diatom Nitzschia pungens as the primary source of domoic acid, a toxinin shellfish from eastern Prince Edward Island, Canada. *Canadian Journal of Fisheries and Aquatic Sciences*, 1989. 46:1203–1215.
2. Wright JLC, Boyd RK, Freitas A, et al. Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. *Canadian Journal of Chemistry*, 1989, 67: 481–490.
3. Perl TM, Bedard L, Kosatsky T, et al. An outbreak of toxic encephalopathy caused by eating mussels contaminated with domoic acid. *The New England Journal of Medicine,* 1990, 322: 1775–1780.
4. Peneva V, Gogov Y, Kalinova G, et al. Application of HPLC method for determination of ASP toxins in bivalve mollusks. Reports and Posters from the Jubilee Scientific Session 110 Years NDNIVMI, 2011, Sofia [In Bulgarian]
5. Lefebvre KA, Robertson A. Domoic acid and human exposure risks: a review. *Toxicon,* 2010, 15:218–30.
6. EFSA. Contam. Scientific opinion of the panel on contaminants in the food chain on a request from the European Commission on marine biotoxins in shellfish — domoic acid. *EFSA J,* 2009, 1181:1-61.
7. EC. Commission Regulation (EC) No 2074/2005. Commission Regulation (EC) No 2074/2005 of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating fromRegulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004.
8. Bates SS, Leger C, Keafer BA, et al. Discrimination between domoic-acid-producing and nontoxic forms of the diatom Pseudonitzschia pungens using immunofluorescence. *Mar Ecol Prog Ser,* 2012, 100:185–95.
9. Bulgarian Executive Agency for Fisheries and Aquacultures. Report on the annual consumption of fisheries and aquacultures and their production in Bulgaria. 2012. Sofia.
10. Petrova E, Stoykov St. Investigations on the marine resources in the Black Sea. 10th national scientific conference of the Bulgarian focal center of EFSA, 2016.
11. SCCWRP & MBC (Southern California Coastal Water Research Project, MBC Applied Environmental Sciences), Santa Monica Bay Seafood Consumption Study. Santa Monica Bay Restoration Project, Monterey Park, CA, 1998.
12. SFEI (San Francisco Estuary Institute) (2000) San Francisco Bay Seafood Consumption Study. SFEI, Oakland, CA, available at www.sfei.org/rmp/sfcindex.htm
13. Mazzillo F, Pomeroy C, Kuo1 J et al. Angler exposure to domoic acid via consumption of contaminated fishes. *Aquatic Biology*, 2010, 9: 1–12.
14. US EPA (1998) Guidance for conducting fish and wildlife consumption surveys. Document No. EPA 823-B-98-007. US Environmental Protection Agency, Washington, DC, available at www.epa.gov/fishadvisories/files/fishguid.pdf
15. Krock B, Tillmann U, John U, et al. LC-MS-MS aboard ship: tandem mass spectrometry in the search for phycotoxins and novel toxigenic plankton from the North Sea. *Anal Bioanal Chem,* 2008, 392 (5): 797-803.
16. Ferriss BE, Marcinek DJ, Ayres D. Acute and chronic dietary exposure to domoic acid in recreational harvesters: a survey of shellfish consumption behavior. *Environment International,* 2017, 101:70-79.
17. Ferrante M, Sciacca S, Fallico R, et al. Harmful algal blooms in the Mediterranean Sea: effects on human health. *Open Access Scientific Reports*, 2013, 2:587.
18. Andjelkovic M, Vandevijvere S, Klaveren JV et al. Exposure to domoic acid through shellfish consumption in Belgium. *Environment International,* 2012, 49:115-119.
19. Ujević I, Ninčević-Gladan Ž, Roje R, et al. Domoic acid — a new toxin in the Croatian adriatic shellfish toxin profile. *Molecules*, 2010, 15:6835–49.
20. Maucher JM, Ramsdell JS. Maternal– fetal transfer of domoic acid in rats at two gestational time points. *Environ Health Perspect,* 2007, 115:1743–1746.
21. Vale P, Botelho MJ, Rodriges SM, et al. Two decades of marine biotoxins monitoring in bivalves from Portugal (1986-2006): a review of exposure assessment. *Harmful algae*, 2008, 7:11-25.
22. Kaniou-Grigoriadou I, Mouratidou T, Katikou P. Investigation on the presence of domoic acid in Greek shellfish. *Harmful Algae,* 2005, 4(4) :717-723.
23. Burger J. Role of self-caught fish in total fish consumption rates for recreational fishermen: average consumption for some species exceeds allowable intake. *J Risk Res*, 2013, 16:1057–1075.
24. Gochfeld M, Burger J. Disproportionate exposures in environmental justice and other populations: the importance of outliers. *Am J Public Health,* 2011, 53–S63.
25. O'Neill CA. Variable justice: environmental standards, contaminated fish, and „acceptable" risk to native peoples. *Stanford Environ Law J.* 2000, 19:46–51.
26. Wells ML. Harmful algal blooms and climate change: learning from the past and present to forecast the future. *Harmful Algae* 2015, 49.